

RESEARCH NOTE

Occurrence of the red abalone *Haliotis rufescens* in British Columbia, Canada

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Abstract: We document the first occurrence of a live red abalone, *Haliotis rufescens* Swainson, 1822, found on the central coast of British Columbia. The initial identification was based on morphological characteristics. Previously, the northern or “pinto” abalone *Haliotis kamtschatkana kamtschatkana* Jonas, 1845 has been the only abalone species considered to naturally occur in the coastal waters of British Columbia. Since hybridization of *H. kamtschatkana* with *H. rufescens* was known to occur, genetic analysis of tissue samples was undertaken to confirm the morphological identification as a purebred red abalone.

Key words: genetics, VERL, hybridization, *Haliotis kamtschatkana*

Along coastal regions of the eastern North Pacific Ocean, from Baja California to Alaska, there are seven abalone *Haliotis* species, all of which coexist in the waters off California and northern Mexico (Geiger 2000, Geiger and Poppe 2000). One species, *Haliotis kamtschatkana*, has been recognized as two morphologically distinct subspecies. These are the northern or “pinto” abalone *Haliotis kamtschatkana kamtschatkana* Jonas, 1845 and the threaded abalone *Haliotis kamtschatkana assimilis* Dall, 1878. Abalone were once sufficiently abundant to support commercial and recreational fisheries in British Columbia (B.C.), Canada, coastal states of the U.S.A., and Baja California, Mexico. Abundances of all abalone species have declined and most abalone fisheries have been closed (Rogers-Bennett 2007). In B.C., the northern abalone was legally listed and protected in June 2003 as threatened under Schedule 1 of the *Species at Risk Act*. The northern abalone has been the only abalone species considered to naturally occur in the coastal waters of B.C. based on surveys spanning 30 years (Sloan and Breen 1988, Adkins 1996, Campbell 2000, Hankewich *et al.* 2008). Herein, we document the identification of an unusual abalone that was discovered by a diver on the north end of Athabaskan Island, central coast of B.C. (52°3.26'N, 128°18.18'W) on 18 December 1998. Vernier caliper measurements of the shell were length 210 mm, width 170 mm, and height 65 mm. The inside edge of the shell was red and the epipodium was black. The abalone, a female based on its green gonad, was tentatively identified as a red abalone, *H. rufescens* Swainson, 1822, according to the morphological characteristics of the shell and epipodium (Cox 1962, Mottet 1978, Haaker *et al.* 1986).

The North American abalone species have overlapping species distributions in the southern part of their ranges,

where at least one of the subspecies of *Haliotis kamtschatkana* has been sympatric with red abalone (Fig. 1). Close genetic relationships among the North American abalone species have been documented in a number of studies on mitochondrial and nuclear DNA sequences, and the underlying evolutionary forces responsible for the relatively recent divergence of the species identified (e.g., Swanson and Vacquier 1998, Geiger 2000, Swanson *et al.* 2001, Galindo *et al.* 2002, 2003). All species recognized on the basis of morphological differences have been confirmed to be distinct on the basis of genetic sequences, with only two subspecies of *H. kamtschatkana* indistinguishable on the basis of molecular analysis (Gruenthal and Burton 2005, Supernault *et al.* 2009).

Hybridization of *Haliotis kamtschatkana* with *Haliotis rufescens* is known to occur (Owen *et al.* 1971, Talmage 1977). Analysis of nuclear DNA sequences, in which one copy of the DNA is inherited from each parent, is useful in distinguishing purebred organisms from first generation (F₁) hybrids. Consequently we examined DNA sequences for the Vitelline Egg Receptor for Lysin (VERL) gene, which differ in nucleotide sequence and/or length among species of abalone (Galindo *et al.* 2003), from the abalone of unknown provenance.

Amplification of the VERL sequence from genomic DNA of the unidentified abalone was carried out according to Supernault *et al.* (2009) with the primers of Galindo *et al.* (2003). Cloned amplified products were sequenced in duplicate and the VERL amplification products from the unknown specimen were sized on an ABI 3730 Sequencer (Supernault *et al.* 2009).

VERL sequence data were analyzed using Sequencher 4.5 (GeneCodes, Ann Arbor, Michigan) and MEGA 4.0 (Tamura *et al.* 2007) and aligned with abalone VERL sequences from GenBank for pink (*Haliotis corrugata* Wood,



Figure 1. Distribution of the northern/threaded abalone *Haliotis kamtschatkana* (indicated by light shading) and the red abalone *Haliotis rufescens* (indicated by dark shading) along the Pacific coast of North America. The location of the red abalone found on Athabaskan Island, B.C., is shown with the circle indicated by an arrow.

1828); green (*Haliotis fulgens* Philippi, 1845); black (*Haliotis cracherodii* Leach, 1814); *Haliotis discus hannai* Ino, 1953; northern/threaded (*Haliotis kamtschatkana kamtschatkana/assimilis*); red (*Haliotis rufescens*); flat (*Haliotis wallacensis* Stearns, 1899); and white abalone (*Haliotis sorenseni* Bartsch, 1940) (Galindo *et al.* 2003, Gruenthal and Burton 2005). MEGA was used to calculate pairwise nucleotide (p) distances for 775 base pairs (bp) of the unknown sequence and

known VERL sequences from northern and red abalone and to construct a multispecies neighbor-joining dendrogram of VERL based on pairwise p distances. The new VERL sequence was submitted to GenBank (accession number EU939887).

We obtained 980 bp of sequence of the VERL gene from the unknown specimen, encompassing 5' to 3', the entire repeats 1 and 2, and 100 bp into repeat 3. The two sequences obtained from the unknown specimen were identical. The pairwise nucleotide (p) distance obtained from the comparison of this VERL sequence with the red abalone VERL sequence was 0.004 (Table 1). In contrast, the pairwise p distances between the unknown VERL sequence and northern/threaded sequences were approximately ten times greater, ranging from 0.034 to 0.053 (Table 1).

Alignment of the new sequence with GenBank sequences of all seven distinct North American and one common Asian abalone species of the North Pacific Ocean, *Haliotis discus hannai*, also confirmed the morphological identification of the specimen as a red abalone, *Haliotis rufescens* (Fig. 2). Comparison of species-specific sites of the VERL amplified product from the unknown individual with sequences from red and northern/threaded abalone provided a match with the red abalone (Table 2). Sizing of the VERL amplification products from genomic DNA extracted from the specimen revealed a single size of 980 bp. Thus, the individual was unlikely to be a hybrid between red and northern abalone. An F₁ hybrid would be expected to possess a VERL sequence from each parental species, and the VERL sequence of the northern/threaded abalone is 1010 bp long.

According to Cox (1962), the natural geographic distribution of *Haliotis rufescens* ranges from Sunset Bay, Oregon to Bahia San Bartolome, Baja California, including the Farallon and Channel Islands. *Haliotis kamtschatkana* ranges from Sitka Sound, Alaska to Turtle Bay, Baja California, but the two subspecies may have disjunct distributions. The northern abalone is distributed from Alaska south to Point Conception in central California and the threaded abalone is distributed from central California to Turtle Bay in Baja California (Geiger 2000). An

Table 1. Pairwise nucleotide p-distances between VERL sequences from the unknown abalone sample and known red and northern/threaded abalone. Values indicative of intraspecific differentiation are in bold.

Species	Red	Unknown	Northern 1	Northern 2	Threaded 1	Threaded 2
Red	—					
Unknown	0.004	—				
Northern 1	0.041	0.053	—			
Northern 2	0.036	0.036	0.004	—		
Thread 1	0.034	0.034	0.005	0.002	—	
Thread 2	0.051	0.053	0.004	0.005	0.007	—

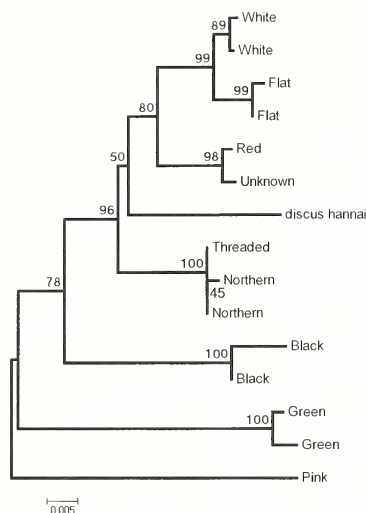


Figure 2. Neighbor-joining dendrogram of VERL sequences from abalone species of the North Pacific Ocean, including one sequence from an individual of unknown species identity. The bootstrap percentage support levels for the tree nodes, based on 1000 iterations, are shown.

overlap of distribution between the red and northern abalone was previously considered to occur only in the southern areas of the northern abalone range.

How the red abalone occurred in B.C. is unknown. Factors that may affect the geographic range of red abalone include climate change and/or human intervention associated

with aquaculture activities. Hobday and Tegner (2002) predicted from simulation modeling of red abalone populations that as water temperatures increase the real red abalone range may extend northward through larval transport. Rogers-Bennett (2007) also suggested northward (and southward) range shifts might be expected in the warmer water abalone species if ocean warming occurs.

Importing an alien species into an area for aquaculture purposes could result in the accidental or intentional introduction of the species into the wild. The red abalone has been a species of interest for aquaculture in Chile (Godoy and Jerez 1998, Pereira *et al.* 2007) and South Africa (Griffiths 2000). Sloan and Breen (1988: 6) reported that "In northern Washington State, *Haliotis kamtschatkana* and *Haliotis rufescens* hybrids were seen a few years after transplantation of California *H. rufescens* (S. Olsen, Washington State Department of Fisheries, pers. comm.)." The introduction of red abalone into Washington State might have provided a convenient launch pad for their northern dispersal under suitable conditions.

Invasions of different biological species into coastal marine habitats have become common in recent years (Ruiz *et al.* 2000, Robinson *et al.* 2005). The implications of an abalone species invasion into B.C. are unknown, but range extensions, based on temporary environmental alterations or human interventions, are often not permanent. On-going quantitative abalone surveys throughout B.C. (e.g., Hanke-wich and Lessard 2008) have not found red abalone, suggesting that this species is probably rare in B.C. There are several anecdotal reports of red abalone shells being observed in B.C. Abalone habitat (Lessard and Campbell 2007) is not considered to be a limiting factor for northern abalone in B.C. (Abalone Recovery Team 2002). Since the northern abalone coexists with other abalone species in California, we assume that there probably is sufficient abalone habitat for a number of abalone species to coexist in B.C. However, natural competition with other marine species,

Table 2. Partial nuclear VERL DNA sequences for red, northern, and threaded abalone compared with the sequence obtained from the unknown abalone found in British Columbian waters. Polymorphic DNA sites are indicated by numbers using the numbering system of Gruenthal and Burton (2005). Letters indicate the nucleotide found in each polymorphic DNA site; periods indicate no change from the top sequence.

Species	Accession number	VERL sequence sites													
		122	207	215	230	278	336	363	375	380	415	423	443	445	498
Red	AF453553	A	G	T	G	C	A	C	G	C	T	C	C	A	G
Unknown	EU939887	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Northern	AY817705	T	A	G	A	G	G	A	C	T	A	A	A	C	A
Northern	AY817704	T	A	G	A	G	G	A	C	T	A	A	A	C	A
Threaded	AY817697	T	A	G	A	G	G	A	C	T	A	A	A	C	A

predation, and reduced fertilization success due to low abalone abundance and density would be expected to keep red abalone scarce in B.C.

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LITERATURE CITED

- Abalone Recovery Team. 2002. National Recovery Strategy for the Northern Abalone (*Haliotis kamtschatkana*) in Canada. Available at: http://www-comm.pac.dfo.mpo.gc.ca/pages/consultations/fisheriesmgmt/abalone/documents/04Abalone_RS.pdf; accessed January 2008.
- Adkins, B. E. 1996. Abalone surveys in south coast areas during 1982, 1985 and 1986. *Canadian Technical Report of Fisheries and Aquatic Sciences* 2089: 72–96.
- Campbell, A. 2000. Review of northern abalone, *Haliotis kamtschatkana*, stock status in British Columbia. *Canadian Special Publication of Fisheries and Aquatic Sciences* 130: 41–50.
- Cox, K. W. 1962. California abalones, family Haliotidae. *California Fish and Game Bulletin* 118: 1–133.
- Galindo, B. E., G. W. Moy, W. J. Swanson, and V. D. Vacquier. 2002. Full-length sequence of VERL, the egg vitelline envelope receptor for abalone sperm lysin. *Gene* 288: 111–117.
- Galindo, B. E., G. W. Moy, W. J. Swanson, and V. D. Vacquier. 2003. Positive selection in the egg receptor for abalone sperm lysin. *Proceedings of the National Academy of Science U.S.A.* 100: 4639–4643.
- Geiger, D. L. 2000. Distribution and biogeography of the Recent Haliotidae (Gastropoda: Vetigastropoda) worldwide. *Bolletino Malacologico* 35: 57–120.
- Geiger, D. and G. Poppe. 2000. *A Conchological Iconography. The Family Haliotidae*. Conch Books, New York.
- Godoy, C. and G. Jerez. 1998. The introduction of abalone in Chile: Ten years later. *Journal of Shellfish Research* 17: 603–605.
- Griffiths, C. L. 2000. Overview on current problems and future risks. In: G. Preston, G. Brown, and E. van Wyk, eds., *Best Management Practices for Preventing and Controlling Invasive Alien Species*. The Working for Water Programme, Cape Town, South Africa. Pp. 235–241.
- Gruenthal, K. M. and R. S. Burton. 2005. Genetic diversity and species identification in the endangered white abalone (*Haliotis sorenseni*). *Conservation Genetics* 6: 929–939.
- Haaker, P. L., K. C. Henderson, and D. O. Parker. 1986. *California Abalone*. State of California Marine Resources Leaflet No. 11.
- Hankewich, S., J. Lessard, and E. Grebeldinger. 2008. Resurvey of northern abalone, *Haliotis kamtschatkana*, populations in southeast Queen Charlotte Islands, British Columbia, May 2007. *Canadian Manuscript Report of Fisheries and Aquatic Sciences* 2839: vii + 39 pp.
- Hankewich, S. and J. Lessard. 2008. Resurvey of northern abalone, *Haliotis kamtschatkana*, populations along the central coast of British Columbia, May 2006. *Canadian Manuscript Report of Fisheries and Aquatic Sciences* 2838: vi + 41 pp.
- Hobday, A. J. and M. J. Tegner. 2002. The warm and cold: Influence of temperature and fishing on local population dynamics of red abalone. *California Cooperative Oceanic Fisheries Investigations Report* 43: 74–96.
- Lessard, J. and A. Campbell. 2007. Describing northern abalone, *Haliotis kamtschatkana*, habitat: Focusing rebuilding efforts in British Columbia, Canada. *Journal of Shellfish Research* 26: 677–686.
- Mottet, M. G. 1978. A review of the fishery biology of abalone. *State of Washington, Department of Fisheries Technical Report* 37: 1–81.
- Owen, B., J. H. McLean, and R. J. Meyer. 1971. Hybridization in the Eastern Pacific abalones (*Haliotis*). *Bulletin of the Los Angeles City Museum of Natural History Science* 9: 1–37.
- Pereira, L., J. Lagos, and F. Raya. 2007. Evaluation of three methods for transporting larvae of the red abalone *Haliotis rufescens* Swainson for use in remote settlement. *Journal of Shellfish Research* 26: 777–781.
- Robinson, T. B., C. L. Griffiths, C. D. McQuaid, and M. Rius. 2005. Marine alien species of South Africa – status and impacts. *African Journal of Marine Science* 27: 297–306.
- Rogers-Bennett, L. 2007. Is climate change contributing to range reductions and localized extinctions in northern (*Haliotis kamtschatkana*) and flat (*H. walallensis*) abalones? *Bulletin of Marine Science* 81: 283–296.
- Ruiz, G. M., P. W. Fofonoff, J. T. Carlton, M. J. Wonham, and A. H. Hines. 2000. Invasion of coastal marine communities in North America: Apparent patterns, processes, and biases. *Annual Review of Ecology and Systematics* 31: 481–531.
- Sloan, N. A. and P. A. Breen. 1988. Northern abalone, *Haliotis kamtschatkana*, in British Columbia: Fisheries and synopsis of life history information. *Canadian Special Publication of Fisheries and Aquatic Sciences* 103: 46 pp.
- Supernault, K. J., A. Demsky, A. Campbell, T. J. Ming, K. M. Miller, and R. E. Withler. 2009. Forensic genetic identification of abalone (*Haliotis* spp.) of the northeastern Pacific Ocean. *Conservation Genetics*. DOI 10.1007/s10592-009-9925-x.
- Swanson, W. J. and V. D. Vacquier. 1998. Concerted evolution in an egg receptor for a rapidly evolving abalone sperm protein. *Science* 281: 710–712.
- Swanson, W. J., C. F. Aquadro, and V. D. Vacquier. 2001. Polymorphism in abalone fertilization proteins is consistent with the neutral evolution of the egg's receptor for lysin (VERL) and positive Darwinian selection of sperm lysin. *Molecular Biology and Evolution* 18: 376–383.
- Talmadge, R. R. 1977. Notes on a California hybrid *Haliotis* (Gastropoda: Haliotidae). *The Veliger* 20: 37–38.
- Tamura, K., J. Dudley, M. Nei, and S. Kumar. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* 24: 1596–1599.

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